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THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

THE DIRECTOR'S TWENTY-SECOND ANNUAL REPORT

1940-41

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Historical Preface

The Walter and Eliza Hall Institute of Research in Pathology and Medicine was founded in 1916. The Trustees of the late Walter and Eliza Hall completed the Pathological Block of the present Royal Melbourne Hospital, and provided an annual payment of £2,500 towards the upkeep of the Institute. In 1925 this was increased to £3,100, and since 1930 has been £3,200 annually. In addition, the Institute has received, since its inception, £500 per annum from the University, the Medical Staff of the Melbourne Hospital having agreed that this portion of its clinical fund should be so used. Since 1925 a further sum of £250 per annum has been provided by the University from interest on the Appeal Fund (1924), together with an annual contribution from the Clinical Research Fund.

In 1926 the late Sir Aaron Danks, then President of the Melbourne Hospital, established an Endowment Fund to support a Biochemical Department. This fund has now reached the total of £8,612. The Edward Wilson Trustees maintained this new department by contributing £1,500 per annum in 1926, 1927 and 1928, and £1,000 in 1929. This Trust also, in 1926, contributed £3,000 for the foundation of an Institute Library, and of this sum £2,000 has been invested as endowment.

The Institute has been supported by annual contributions from the Felton Bequest Committee (since 1928), the Trustees of the late Anthony Mackie (since 1933), and the Trustees of the late T. J. Sumner (since 1929). The Trustees of the late Alfred Edments have also helped us since 1937.

The Institute has received substantial support from the Department of Health of the Commonwealth Government; in the years 1927, 1928, 1929, 1930 and 1931 a total of £10,650 was received for special researches. In 1934 the Commonwealth Government entered into an agreement with the Rockefeller Foundation that each would contribute to the Institute the sum of £1,000 per annum for the ensuing three years to support work on Virus diseases, particularly those affecting the central nervous system. In 1937 the Rockefeller Foundation and the Department of Health of the Commonwealth Government agreed to continue their support of this work for a further two years. The National Health and Medical Research Council have provided additional funds during 1938, 1939, 1940, and 1941, and Mr. E. Alex. Cato has generously arranged to contribute £1000 per annum for the five years' period from 1st January, 1939, to 31st December, 1944.

In 1932, by the will of the late Mrs. L. E. W. Carty, of Brisbane Hill, Hamilton, the Elsie Marion Carty Fund was founded. Mrs. Carty willed to the Institute a portion of her estate and a residuary interest therein to establish this Fund. It was her wish that the income should be used, primarily, for the assistance of promising research workers.

From 1934 to 1937 Mr. A. M. Nicholas gave an annual sum of £250 towards the salary of the Assistant Director, and Mrs. Nicholas generously continued this contribution in 1938 and 1939.

In 1936 the Carnegie Corporation of New York paid the travelling expenses of Dr. W. Feldberg and his family to Australia, and contributed in addition 4,500 dollars, which, with £1,100 collected by Mr. Reuben Hallenstein, made it possible to maintain this worker for two years.

The establishment of the National Health and Medical Research Council in 1937 has aided greatly in the development of this and other institutes for medical research in Australia.

It was finally decided in May, 1938, to rebuild the Royal Melbourne Hospital on the new site in Parkville. The Committee of Management subsequently accepted the responsibility for a new Institute building, the cost of which was estimated at £45,000. Towards this amount Mr. G. R. Nicholas and the family of the late Mr. A. M. Nicholas—Mrs. A. M. Nicholas, Mr. Maurice Nicholas, and Mrs. William Gluth—contributed £20,000. Mr. Russell Grimwade and the Broken Hill Proprietary Company Limited gave each £1000, and a few other gifts have brought the special fund for this purpose to a total of £22,075.

To provide essential space in the present building for war work, the Research Biochemical Department was moved into the second floor of the new building in May, 1941.

The Institute is controlled by a Board consisting of representatives of the Walter and Eliza Hall Trust, of the University of Melbourne, and of the Committee of Management and Medical Staff of the Royal Melbourne Hospital. During the Great War Professor Sir Harry Allen acted as Honorary Director. In 1919 Dr. S. W. Patterson was appointed first Director; in April, 1923, he resigned, and was succeeded by Dr. C. H. Kellaway in August, 1923. During the interval Mr. H. Dew acted as Director.

The Director's Twenty-Second Annual Report

TO THE BOARD

OF THE

Walter and Eliza Hall Institute of Research in Pathology and Medicine

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JULY, 1941.

The Institute this year has been able to increase its contribution to the war effort. I have been a member of the Medical Directorate at Army Headquarters during the whole of the period under review. During the year over 42,000 members of the A.I.F. and R.A.A.F have been blood grouped, and over two hundred medical officers and nurses have been vaccinated and inoculated. In addition blood grouping and blood tests have been carried out on a large number of emergency donors for the Red Cross Transfusion Service. Medical officers and laboratory assistants have been trained in pathology and other army personnel in blood transfusion methods. Nearly 1000 sterility tests have been done on catgut for service use. The researches of the Institute have so far as possible been concentrated on problems of war-time importance. Dr. Burnet has continued his studies on immunization against influenza. Bacteriophages are being prepared for the laboratory diagnosis of dysentery. Work is in progress on methods of preparing pyrogen-free water, on the concentration and chemical drying of serum, on pharmacologically active substances in human serum, on micro-methods of testing for arsenic in biological fluids, on the preparation of complement in dried form, and on the preparation and testing of "amnioplastin." A full account of these activities is given later in this report.

Through the generosity of Mr. A. P. Adams, who has contributed £1000 to form a special war research fund, additional money for research of this kind has been provided. This will be expended not only in this Institute, but in University departments and in other institutes on the advice of Major-General Downes, C.M.G., Professor MacCallum, and myself. So far allocations have been made for the preparation of special agglutinating sera and emulsions at the Institute of Medical and Veterinary Research, Adelaide, for materials for the preparation of dried complement (guinea-pig serum) for use in the Middle East, for the study of pharmacologically active substances in human serum, and for the preparation of bacteriophages for the diagnosis of dysentery. For this last work Miss D. M. Langley has been appointed for one year to work under Dr. Burnet's direction.

Expansion of the work of blood grouping and blood tests for the Emergency donor service of the Victorian branch of the Red Cross Society, the appointment of a second whole-time worker for this purpose, and the need for training V.A.D.'s in blood transfusion methods have necessitated the provision of more space in the Institute. This has been obtained by moving the Research Biochemical Department into the new building, on the Parkville site. Although the interior of the building was in an unfinished condition, the builders, Messrs. J. and R. Seccull, made the whole of the second floor ready to receive these workers in less than three weeks. Except for hot water and steam, which will not be available until the hospital is moved, all other facilities are provided. This move took place early in May.

Dr. Drevermann, who was a Junior Research Fellow of the National Health and Medical Research Council, enlisted last year, and was called up to take charge of the bleeding of the emergency donors for the preparation of serum for overseas. Dr. Lucy Bryce has been appointed Pathologist to the military hospital at Heidelberg.

The war, and the fact that our honorary Editor, Major Colin W. Ross, has been engaged in full-time service as a member of the Medical Directorate at Army Headquarters, have caused unavoidable delays in the publication, which was anticipated in last year's report, of the new series of Institute monographs. The first by Dr. F. M. Burnet, on the production of antibodies, is in page proof; the second, by Dr. E. Graeme Robertson, on Encephalography, and the third by a number of authors on the technique of blood transfusion are well advanced. It is hoped that their publication will not be much longer delayed.

VIRUS DEPARTMENT.

Influenza Virus Infection of Chick Embryos by the Amniotic Route.

A large proportion of the work on influenza virus carried out during the year has developed out of the discovery reported in the last annual report that all types of influenza virus will multiply readily and produce characteristic changes when inoculated into the amniotic cavity of developing chick embryos. The method seems likely to become one of considerable practical importance as (1) it makes it possible to carry out detailed experiments with virus strains which have not been modified by animal passage, (2) it is an excellent method for the primary isolation of influenza virus from human sources, and (3) it provides a source of high titre sterile virus of any desired strain for vaccine or other uses. Detailed studies on these and other aspects are described in the following paragraphs.

Pathology of Amniotic Infections.—Influenza virus introduced into the amniotic cavity enters the trachea through swallowing and other movements of the embryo, and eventually reaches the finer tubules (parabronchi). The epithelial lining of the parabronchi, and of the saccules which bud off to form eventually the pulmonary air capillaries, is the chief site of virus proliferation and cellular damage. With a virulent strain there is complete desquamation of the respiratory epithelium, and great disorganisation of lung structure. The desquamated epithelium passes into the trachea, where its presence is used experimentally as the chief indicator of infection, and thence to the amniotic cavity. During the course of inoculation or subsequently the lining of the allantoic cavity becomes infected, and multiplication of virus occurs. As a result both amniotic and allantoic fluids show a high virus titre when removed after two or three days. The lungs contain much more virus than any other of the organs of the embryo. During the early passages the infection is rarely fatal in less than four days, and late sections of the lungs show well marked healing processes, though with complete loss of the normal structure. After about ten amniotic passages the infection becomes more rapidly lethal, most embryos receiving moderately large doses dying within three days. A characteristic feature of these fatal infections is the disappearance of the amniotic fluid, leaving the embryo tightly bound up in a contracted amnion.

Isolation of Virus from Human Throat Washings.

Although there was little influenza in Melbourne during the winter of 1940, there were sufficient opportunities to show that the method was very suitable for primary isolation of virus from human throat washings. Seven isolations have been made from human material, and several from experimentally infected ferrets and monkeys. The throat washings were obtained in the usual way, and gradocol membrane filtrates prepared. Several eggs were inoculated amniotically from each filtrate with about 0.25 cc. of fluid. After three days' incubation one embryo was examined, and if this was negative the remaining eggs were left one or two days longer. Fluids collected from eggs showing the usual signs of infection were proved to contain influenza virus by inoculating them intranasally in mice. They produced no symptoms, and only minimal pulmonary lesions, but the mice were found to be solidly immune to mouse passage virus of related antigenic type fourteen days later. A point of particular interest was the demonstration that two primary filtrates which failed to induce any temperature rise in ferrets gave typical infections amniotically. Although in this work ferrets were used to confirm the findings and to provide sera for the serological analysis of the strains encountered, it has been shown by Dr. Burnet and Miss Foley that it is quite practical to isolate and characterize immunologically an influenza virus strain without the use of ferrets.

Changes in Virulence on Repeated Amniotic Passage.

Several strains have been submitted to serial amniotic passage, and their virulence for various test-organisms examined at intervals. The most interesting of these experiments concerned the strain "R" isolated in July, 1939. An amniotic passage series was commenced with first passage ferret material preserved in glycerol saline at low temperature for a year. A mixture of first and second amniotic passage material was shown to produce typical influenza in three of four low-antibody human volunteers, the forty-second passage gave material which was inoculated intranasally in fourteen low-antibody volunteers, only two suffered from definite symptoms (of minor character), six including these two showed a definite antibody rise, and eight showed no evidence of infection. This represents the first unequivocal evidence that human virulence does fall progressively with passage in an abnormal host. The same passage series showed a gradual fall in ferret virulence. The strain was initially of very low pathogenicity for mice; it developed up to about the fortieth passage an increased virulence, which then gradually diminished. Changes in the effect of the infection on the embryo were also of interest. With nearly all strains the early passages allowed most of the embryos to survive at least four or five days, but after about ten passages the virulence increased, so that even with very small inocula death of the embryo usually occurred within three days. This lethal effect on the embryo was entirely distinct from that of highly adapted chorioallantoic passage strains which produce haemorrhagic lesions in brain and muscles. The active amniotic strains produce no gross lesions in the embryo apart from those in the lungs, and one of the causes of death may be the disappearance of almost all amniotic fluid and constriction of the embryo by the contracted amnion.

Immunological Work.

In view of the possibility that strains of influenza virus might be obtained which failed to produce recognizable infections in ferrets or mice, a study was made of the practicability of serological tests by the amniotic method. Virus strains whose activity could be tested in mice as well as in chick embryo were used, and the mixtures with immune serum inoculated into mice intranasally and amniotically in eggs. With strains unadapted to the egg, i.e., mouse lung virus, the results were similar in the two test objects, except for an occasional ''take'' in the egg of a mixture inactive in mice. With egg virus well adapted to amniotic passage there was a striking discrepancy, mixtures ''greatly overneutralized'' for mice being capable of inducing infection amniotically. The main effect of the immune serum was to prolong the survival time of embryos inoculated with the serum-virus mixtures.

The Immunizing Capacity of Unadapted Virus for Mice.

The availability of a method for the production and titration of unmodified influenza virus has made it possible to carry out several types of experiment not otherwise practicable. In many respects the reaction of mice to unmodified human influenza virus might be expected to be very similar to that of human beings to virus attenuated by passage through a foreign host. For this reason a good deal of attention has been paid to the response of mice to unadapted virus given intranasally in the form of pooled fluids from eggs infected amniotically. The work has been limited to some extent by the fact that the only unadapted strains in our possession are all of one antigenic type. The present indications are (1) the minimal immunizing dose varies with the strain, but is always 1000 or more times the minimal infecting dose by the amniotic route; (2) that when undiluted virus-containing fluids of high titre are used as immunizing agents small lesions are often produced, and irrespective of whether lesions are or are not visible a high degree of resistance to strains of homologous and heterologous (W.S. and Swine) antigenic type is produced; and (3) that with small immunizing inocula the immunity produced is highly specific for the homologous strain.

Human Immunization Against Influenza.

Up to the present the only effective methods of immunization against virus diseases of man, Jennerian vaccination and immunization against yellow fever, have involved the use of living attenuated virus given by an approximately natural route. In England and America attempts at immunization against influenza are being made by the subcutaneous inoculation of various types of antigen. There are both theoretical and practical reasons for thinking that a method of inducing subclinical infection by the natural route would be a better method of immunization if a suitably attenuated strain could be made available. In previous years Dr. Burnet and Miss Lush showed that attenuated strains could be developed by passage on the chorioallantois, and as mentioned in an earlier paragraph this holds also for amniotic passage. With the new method it should theoretically be possible to provide material of any desired degree of virulence. During the current year a number of experiments on human volunteers have been made in an attempt to explore more directly the possibilities of this type of immunization. In the first experiment two administrations of strains attenuated by chorioallantoic passage were followed by a test inoculation, carried out in isolation, with what was considered to be unmodified human influenza virus. As a result of this inoculation three of the four subjects in the group with very low antibody contracted clinical influenza, and all four showed a rise of antibody to a high level. The eleven subjects with relatively high initial antibody showed one transient rise of temperature with antibody rise, and two rises of antibody without symptoms. This experiment indicated first that well-attenuated strains known to be capable of immunizing mice and ferrets were unable to provoke an antibody rise or to protect against a virulent strain in human subjects with low antibody titre. In the second place it showed that under the conditions of experiment typical influenza was produced only in subjects with very low antibody titre, and thirdly it indicated that with only one passage through ferret and two amniotically in eggs a strain of influenza had retained its full power to produce human symptoms. This experiment was unsuccessful in demonstrating any immunity from the administration of highly attenuated strains, but it provided a valuable starting point for further work.

The period of isolation in this experiment was spent in very comfortable circumstances, through the kindness of Dr. and Mrs. Ivan Maxwell in making their seaside house available. We are also very grateful to Mrs. N. Hamilton Fairley and Mrs. F. M. Burnet for their help in arranging for provisioning and the other domestic details. The expenses of this experiment were defrayed by a grant from the Marion Carty Fund.

Subsequent work on human immunization has taken the form of testing various partly attenuated strains on volunteers chosen for their low initial antibody level. We have been able to enlist the co-operation of a majority of the students at the various hospitals, and a rough test of antibody level was made on all volunteers. For this purpose a single drop of citrated blood tested against a suitably diluted virus allowed a differentiation into groups with high, average, and low antibody. Only those with antibody definitely below the average level were used for further tests. This work is still incomplete. The results have been consistent in showing first that with attenuated strains mild symptoms are produced only in persons with very low antibody; these show an antibody rise as do a proportion of those who show no symptoms, but more than half have failed to show any response whatever. In most experiments the antibody rises have been much smaller than those following frank infection, but in the most recent series, using an attenuated "W.S." strain, three of six subjects showed a hundredfold increase of titre.

It is as yet by no means certain what determines the incidence of clinical influenza during an epidemic, and the possible value of our immunization procedures with attenuated living virus seems to hinge largely on whether there is any correlation between the persons who "take," i.e., give an antibody response and those who, when exposed to epidemic conditions, would develop influenza. If the two groups should coincide the method might protect almost all those who needed protection. This assumption is a reasonable one, but there is no direct evidence that it holds. The possibility of preparing a complex vaccine with representative attenuated strains of each main antigenic type of virus is also being studied. In view of the known complexity of certain influenza epidemics, practical success would probably demand the use of a complex immunizing agent.

Influenza Infections in Monkeys.

In the hope that the reactions of monkeys might in some respects throw more light on human reactions to influenza virus than do those of the usual experimental animals, Dr. Burnet has carried out a series of experiments with cynomologus monkeys. No symptoms are produced when the virus is given intranasally, but with intratracheal injection a definite one to three day temperature rise occurs, and there is a sharp antibody response. A point of interest in these experiments is the demonstration that repeated intratracheal injections can each produce a considerable antibody rise, indicating that reinfection occurs on each occasion. Two animals given repeated inoculations of human strains have developed swine type antibody. These experiments are being continued.

After inoculation with one strain of virus (egg adapted "W.S.") a monkey developed pneumonia and died in eight days. The lesions had all the characteristics of a pure virus pneumonia, and the strain was re-isolated from the lungs. With this strain a series of alternate monkey and amniotic passages is being carried on. Intratracheal inoculation with high titre egg virus is now regularly producing fatal pneumonia from the lesions of which only the virus can be isolated. No symptoms or lung consolidation is produced in immunized animals, so that the condition is definitely a pure influenza virus pneumonia. Dr. Barbara Wood is collaborating in radiographic study of the lesions, and experiments are planned to elucidate the pathogenesis of the condition and to determine the effectiveness of various methods of immunization.

The Multiplication of Influenza Virus in the Allantoic Cavity.

With a view to the possibility that large amounts of virus might eventually be required for the preparation of immunizing material, attempts have been made to simplify the amniotic inoculation technique. An extremely simple and effective method has been developed from the observation that all types of influenza virus so far tested will multiply in the lining of the allantoic cavity, and give high titre virus in the allantoic fluid. Eggs can be inoculated by a needle inserted through a very small cut in the shell in less than 1/10th the time required to complete an amniotic inoculation. Despite the fact that by this method 8 to 10 cc. of fluid active to a titre of 10^7 to 10^8 can be obtained from each egg there is surprisingly little effect on the embryo which, if left, will hatch normally. The details of the method and the immunizing capacity of fluid taken at various periods are being studied.

Influenza Virus B.

The various strains of human influenza virus isolated up to 1940 were all immunologically related, but last year Francis described a new strain which, although generally similar to former strains, is antigenically quite distinct. The available evidence is that the new type "influenza B" has been responsible for several typical influenza epidemics in America since 1936. The new virus is very difficult to isolate, and so far only one strain has been described. Through the kindness of Dr. Francis this strain has been received by Dr. Burnet, and it has already been shown that B type infections have been occurring in Australia in recent years. The Francis strain produces typical infections when inoculated amniotically, and if this should be characteristic of B type strains in general, the method should be by far the most convenient means of primary isolation.

Action of Psittacosis and Laryngotracheitis Viruses on Amniotic Inoculation.

Preliminary work with all the viruses available showed that, apart from influenza strains, only psittacosis and laryngotracheitis viruses produced significant lesions after amniotic inoculation in chick embryos. Miss Foley and Dr. Burnet have made a fairly extensive study of these infections. Neither virus shows the intense activity of influenza viruses. Psittacosis virus can be passaged indefinitely, and the tracheal fluid constantly contains L.C.L. bodies. In sections the epithelium of the larger bronchial divisions is mainly affected, and large cytoplasmic colonies of L.C.L. bodies can be seen in some of the superficial cells. Infection of the parabronchi is variable and usually slight. The intensity of reaction is more or less proportional to the amount of virus injected, and with high dilutions very little effect is apparent although the virus can be shown to have multiplied.

Laryngotracheitis virus sometimes produces very extensive lesions, involving practically the whole of the respiratory epithelium, but there are great irregularities from one embryo to another, and continued passage could only be maintained with difficulty. The lesions were of the characteristic proliferative-necrotic type in intranuclear inclusions. Even in the most extensive lesions the infected cells tended to remain in position, and there was none of the complete disorganisation of lung tissues characteristic of influenza virus lesions.

It may be added that on two occasions out of about a dozen attempts fowlpox virus infections have been produced in the chick embryo lung.

Influenza in Military Camps.

In the winter of 1940 the prevailing respiratory infection in Victorian camps took the form of an acute pharyngitis and laryngitis (Puckapunyal throat). This infection did not resemble typical influenza, and a number of attempts to isolate influenza virus were unsuccessful. In the later months of winter more influenza-like infections occurred, and Major G. V. Rudd, A.A.M.C., investigated several groups of such cases. His findings were that of three small groups of cases studied in July, August, and September respectively only the August group showed evidence of infection by influenza virus A. Of four persons in this group influenza virus was isolated from one, and two others showed a characteristic rise of antibody.

Although the September cases were clinically similar, and seemed to result from the same epidemic, they showed no serological evidence of infection with type A influenza virus. A recent examination of some of the sera left by Major Rudd suggests that influenza B infection also occurred in this camp during 1940.

Low Temperature Cabinet for Storage of Viruses.

Recent work in America has shown that the most satisfactory method for the maintenance of viruses without change in virulence is by freezing at —78° C., a temperature which can be maintained with solid CO_2 (dry ice). We are very grateful to a sub-committee of the Institute of Refrigerating Engineers (Messrs. H. F. Coleman, E. Gordon, and F. Anderson) for their help in designing a suitable cabinet for this purpose. The cabinet has been manufactured, and is in commission. It should be of particular value for maintaining stocks of attenuated influenza virus strains at the desired level of virulence.

Poliomyelitis.

A certain amount of work on experimental poliomyelitis in cynomolgus monkeys has been carried on during the year, partly by Dr. Burnet and partly by Major T. C. Backhouse. This has been directed wholly toward elucidating the route by which virus administered orally reaches the central nervous system. Since at the present time there is a strong swing of opinion toward the view that poliomyelitis is primarily an intestinal disease, these experiments may throw some light on the pathogenesis of the human disease. The evidence obtained is compatible with the view that virus administered orally can infect and multiply in the cells lining some portion of the pharynx and in the intestinal mucosa. From these situations it passes to the corresponding deep cervical and mesenteric lymph nodes, and reaches the central nervous system mainly by autonomic nervous pathways. The earlier part of this work has been published, but the later experiments by Major Backhouse are still unpublished. His work added three further examples of infection by the pharyngeal route, and in two of five experiments he succeeded in infecting monkeys with virus administered by stomach tube. After pharyngeal infection the deep cervical lymph nodes contained virus in two instances, and the mesenteric lymph nodes also in two of three tests. Virus was also isolated from scrapings of the washed intestinal mucosa. A finding of particular interest was obtained from a monkey prostrate ten days after intracerebral infection. From this animal the virus was isolated from both the washed intestinal mucosa and a mesenteric lymph node. This may probably be interpreted as a secondary infection of the intestinal mucosa.

Dysentery Bacteriophages.

With the possibility in mind that a series of specific bacteriophages might prove the most convenient method for the rapid typing of dysentery bacilli, and perhaps therefore be of value in elucidating epidemiological aspects of bacillary dysentery, work along these lines has been continued at irregular intervals through the year. As the discovery of suitably specific bacteriophages is largely a matter of chance, the work has been slow. Miss Langley has now taken up this problem, and through the co-operation of Dr. Jago, of Mont Park, is obtaining material from mental hospitals where Flexner infections are more or less endemic. A fairly satisfactory set of bacteriophages has been built up, but there are certain inconsistencies in the results which need to be cleared up before the method could be of any practical value.

Serological Classification of Invertebrates.

Miss Clark, who has been appointed secretary of the Virus Department, was previously engaged in the study of Australian fresh water crustacea at the National Museum. This group is very suitable for the study of species and genus relationships by immunological methods, using the precipitin test with serum from immunized rabbits. Miss Clark is therefore undertaking this work, and has already shown that the three genera, *Euastacus, Engaeus,* and *Cherax,* can be clearly differentiated by serological methods. The study of finer differences using absorption methods is at present under way.

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''Poliomyelitis IV. The Spread of Poliomyelitis Virus in Cynomologus Monkeys, with Particular Reference to Infection by the Pharyngeal-Intestinal Route,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1940, 18, 361.

F. M. Burnet and M. Foley:

''The Results of Intranasal inoculation of modified and Unmodified Influenza Virus Strains in Human Volunteers.'' ''Medical Journal of Australia, 1940, 2, 655.

''Two Methods for the Detection of Influenza Virus in Human Throat Washings Without the Use of Ferrets,'' ''Medical Journal of Australia,'' 1941, 1, 68.

''Influenza Virus Infections of the Chick Embryo by the Amniotic Route. III. Changes in the Activity of Influenza Virus on Continued Amniotic Passage,'' ''Australian Journal of Experimental Biology and Medical Science'' (in the press).

''Infections of the Respiratory Tract of Chick Embryos by the Viruses of Psittacosis and Infectious Laryngotracheitis—a Comparison with Influenza Virus Infections,'' ''Australian Journal of Experimental Biology and Medical Science'' (in the press).

G. V. Rudd and F. M. Burnet:

''Intranasal Infection of Mice with the Virus of Psittacosis,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1941, 19, 33.

G. V. Rudd:

''Influenza Epidemic at Puckapunyal,'' ''Medical Journal of Australia,'' 1941, 1, 7.

I. M. Mackerras, M. J. Mackerras, and F. M. Burnet:

''Experimental Studies of Ephemeral Fever in Australian Cattle,'' Council for Scientific and Industrial Research, Bulletin 136, 1940.

DEPARTMENT OF PHYSIOLOGY.

Dr. Trethewie, who was a Junior Research Fellow of the National Health and Medical Research Council, resigned this appointment early this year to become Lecturer in Physiology at the University, and Dr. G. Reid was appointed a Junior Research Fellow in his place.

During the period under review Dr. Trethewie, Dr. Reid, and I have continued our studies on the effects of tissue injury by the toxins of the anaerobes. We have completed the investigation of the toxins of Cl. welchii types A, B, and C and of Cl. septique. Recently Mr. Holden, Dr. Reid, and I have taken up the study of those pharmacologically active substances which are liberated when blood clots, and which are said to be present in human and other sera.

Dr. Trethewie has investigated the use of ''amnioplastin'' in animals, and has continued this work in the University Department of Physiology. He has also carried out some experiments on the implantation of kangaroo tendon in muscle and tendon.

Tissue Injury by the Toxin of Cl. welchii, Type A.

This toxin fails to cause the liberation of histamine or of other muscle-stimulant substances from perfused organs, but sets free pigment, coagulable protein, active adenyl compounds, and an enzyme capable of inactivating these latter from the perfused liver of the rabbit. Adenyl compounds and the inactivating enzyme are also liberated from perfused hind limb preparations. This toxin causes no immediate direct liberation of adrenalin when it is injected into the arterial blood supply of the suprarenal glands of the cat, but in experiments on the intact guinea-pig considerable loss of the residual adrenalin in the suprarenal glands was observed, and this was found to be a specific effect not occurring when neutralised toxin was injected. It is, however, unlikely to result from direct action of the toxin on the glands.

In confirmation of other workers this toxin was found to cause contraction of various types of smooth muscle, the isolated jejunum of the guinea-pig, the uteri of the virgin guinea-pig and rat, the bronchial musculature of the guinea-pig, the pulmonary vessels of the rabbit, and the coronary vessels of the rabbit and cat. This specific contraction of plain muscle caused by the toxin cannot be attributed to histamine, because there is no evidence of the liberation of this substance from perfused organs. The specific contraction observed in the uterus of the rat (which is relaxed by histamine) supports this belief. The evidence suggests that the contraction is a direct effect of the toxin.

Tissue Injury by the Toxins of Cl. welchii, Types B. and C.

These two toxins, when injected intravenously in large doses, have similar effects on the circulation of cats, both causing a rise in pressure in the pulmonary artery and a terminal steep fall in systemic pressure often accompanied by heart block and invariably by a sharp rise in the venous pressure. The first effect with small doses of the toxin of Cl. welchii, type C, is a prolonged rise of systemic blood pressure.

The toxin of Cl. welchii, type B, causes the liberation of histamine from the perfused lung of the cat, but fails to do so from the perfused lung of the guinea-pig. The toxin of Cl. welchii, type C, causes the liberation of a slow reacting muscle-stimulant substance, but not of histamine from both preparations. Both toxins cause the liberation of pigments and of adenyl compounds from the perfused liver of the rabbit, and also of a heat labile agent which inactivates these latter compounds. Both toxins cause contraction of the isolated jejunum of the guinea-pig and of the uteri of the virgin guinea-pig and rat. These responses were shown to be specific, except that of the isolated uterus of the guinea-pig to type B toxin, in which a histamine-like impurity was present. It is not surprising that B and C toxins exhibit a general similarity in their reactions, since apart from the presence of some epsilon fraction in B, they have a similar antigenic composition. In both cases this is somewhat complex. It seems likely that the epsilon fraction is responsible for the liberation of histamine by Cl. welchii, type B, because D toxin, which is largely composed of this antigen, has been shown to liberate histamine from perfused tissues. If this is so, the absence of epsilon fraction from C may be held to account for failure by this toxin to liberate histamine from perfused organs. In their action on smooth muscle these toxins exhibit another difference, the contractions after C toxin requiring a very much longer time for relaxation. This may be related to the liberation of a slow-reacting muscle-stimulant substance by C toxin or may be a direct effect of the toxin itself, in which case it may depend upon the presence of some as yet undescribed antigenic constituent.

Tissue Injury by the Toxin of Cl. septique.

This toxin causes the liberation of histamine from the perfused lung of the guinea-pig, and of adenyl compounds and an inactivating enzyme from the perfused liver of the rabbit. It also causes the liberation of adrenalin by direct action upon the suprarenal glands. Its effect upon plain muscle resembles that of Cl. welchii, type C, relaxation being extremely slow. Relaxation cannot be hastened by repeated administration of adrenalin, though the contracted muscle is sensitive to its action and relaxes well in response to small doses of this substance. When the drug is removed the muscle at once returns to its previous state of contraction. The administration of large doses of antitoxin also fails to hasten relaxation, though the contraction itself is wholly specific and is not given by neutralized toxin. A change in the muscle which does not interfere with its reaction to adrenalin, though it impairs the response to histamine, is set up specifically by the action of toxin. We have no evidence as to whether this is a direct effect, or is caused by the presence of some slow-reacting muscle-stimulant substance, though our perfusion experiments gave no indication of the formation or liberation of such a substance.

The circulatory effects of the toxin of Cl. septique were studied in the cat and in the rabbit. In the cat, after large doses, death takes place rapidly with constriction in the pulmonary circulation and rapid failure of the heart. The failure is also attributable partly to toxic action on the heart muscle and partly to constriction in the coronary circulation. In smaller doses the toxin somewhat irregularly causes oedema of the lungs with loss of fluid from the circulation.

In the rabbit, which is much more susceptible than the cat, the first effect of the toxin is a rise of systemic blood pressure, which is interrupted by temporary or final failure of the heart. The toxin has a transient constrictor effect on the peripheral vessels, but the secondary rise of pressure with quickening of the heart beat which occurs during recovery from the early cardiac failure is a central effect. It is followed after a few minutes by a gradual and final fall, which is not caused by direct peripheral action of the venom, but is constantly associated with constriction in the pulmonary circulation. Oedema of the lungs was rarely observed in these acute experiments.

The Extraction of Adenyl Compounds from Tissues.

Dr. Trethewie and I studied the extraction of active adenyl compounds from tissues, and found that the most satisfactory method was to grind them up with finely divided quartz in Tyrode solution and bring the extracts immediately to the boil. This method gave higher values than had been obtained by other workers, who also had estimated active adenyl compounds biologically. This we attributed to more complete extraction from the tissues.

Pharmacologically Active Substances in Human Serum.

The use of human serum in large dosage (2 to 3 pints) for the resuscitation of persons suffering from shock or haemorrhage raises the question of the importance or otherwise of the pharmacologically active substances which appear in serum when blood clots, but which are not present in whole blood. Mr. Holden, Dr. Reid, and I are investigating the conditions under which these active substances are produced in minimum amounts and those under which they disappear from the serum. So far our results suggest that, when the formed cells are separated from the plasma of oxalated blood before the calculated amount of calcium is added to produce clotting, the amount of substances which cause contraction of isolated smooth muscle preparations are much less than when serum is removed from blood shed without any anticoagulant being present and allowed to clot. The former method is that used by the Commonwealth Serum Laboratories in the preparation of human serum for transfusion. The presence of adenyl compounds in serum is well attested, but it seems likely that the source of these substances is the formed elements of the blood. We have so far been unable to demonstrate even traces of active adenyl compounds in serum prepared by either of the methods described above.

Amnioplastin.

In conjunction with workers of the Pathological Laboratories of the Women's Hospital Miss F. E. Williams has prepared amnioplastin, sterile human amniotic membrane, for use for the prevention of adhesions in man. For the sterilization of this product we found it convenient to use dry heat instead of steam under pressure, as advocated by Penfield. Recently Penfield has drawn attention to the necessity either of boiling these membranes or of removing all fat from them by means of solvents.

Dr. Trethewie has studied the results of implantation of this human amniotic membrane inside the skulls of animals. The membrane, which had been defatted or boiled before use, was found to give exactly the same results as our dry sterilized membrane. Provided that the amount of damage to the cerebral cortex was reasonably severe, in no case did the preparation fulfil the claims made for it. These tests have been done partly at the Hall Institute and partly in the Department of Physiology of the University. The expenses of the preparation of amnioplastin have been defrayed from a grant of £100 from the Royal Australasian College of Surgeons.

Experiments on Kangaroo Tendon.

Dr. Trethewie has studied the fate of kangaroo tendon implanted in the muscle (sacro-spinalis) and tendon (hamstrings) of the guinea-pig. The material was prepared for orthopaedic work, and sterilized by the iodine method by Miss F. E. Williams. In muscle the kangaroo tendon persists almost unchanged in appearance for as long as three months. It undergoes some loss of tensile strength which falls to 20-25% of its original value during the first month, and to 10% at the end of three months. Implanted in tendon it is digested, disappearing almost completely in two to four weeks. It is possible that the rapid digestion in tendon may depend upon contact with synovial fluid.

C. H. Kellaway and E. R. Trethewie:

> "A Note on the Extraction of Adenyl Compounds from Tissues," "The Australian Journal of Experimental Biology and Medical Science," 1940, 18, 265.
>
> "Tissue Injury by the Toxin of Cl. welchii, Type A," "The Australian Journal of Experimental Biology and Medical Science," 1941, 19, 17.
>
> "The Injury of Tissue Cells and the Liberation of Pharmacologically Active Substances by the Toxins of Cl. welchii, Types B and C" (in the press).

BIOCHEMICAL DEPARTMENT.

During the year under review Mr. Holden had to devote an increasing proportion of his time to matters not connected with his own work but which are, at the moment, of greater urgency. He completed his studies on the protoporphyrin complexes of cobalt, zinc, nickel, and copper. He then studied in more detail the compounds of cobalt-protoporphyrin, and succeeded in reducing cobalti-protoporphyrin and its globin compound to corresponding cobalto-protoporphyrin compounds. He investigated cobalti-protoporphyrin compounds with potassium cyanide and with bases of the iminazole group. These are interesting for the fact that their "soret" band moves from the ultraviolet to the violet region of the spectrum. Little is as yet known of their constitution. A paper awaits publication.

Fearing a possible shortage of asbestos bacteriological filters, Mr. Holden studied their composition and succeeded in preparing suitable substitutes for those commercially available. In this connection grateful acknowledgment is made of the help afforded by the Directors and the Research Staff of the Australian Paper Manufacturers.

Mr. Holden has also helped in the work, mentioned elsewhere in this report, on the drying of serum under aseptic conditions. Since January he has tried various methods of drying complement (guinea-pig serum) for transport, and has selected the Flosdorf-Mudd method which appears to give good results. He has also dried, with this apparatus, small amounts of sera for pharmacological studies with Dr. Reid and myself.

The work on the slow-reacting substance has been continued, but has not made any great progress. The best extracts so far prepared are about 30 times as active as the crude preparations of envenomed egg yolk with which the work was started. All attempts, physical or chemical, to effect a further removal of the inactive constituents of the fraction have proved unsuccessful.

During 1940 Mr. Rawlinson was able to continue his work on metallo-proteins and substances containing metals in complex combination with the aid of the magnetic balance. He studied the effect of various solvents on the susceptibility of haemin. He prepared potassium molybdocyanide and molybdicyanide in the pure state, and measured their magnetic susceptibilities. His work on haemocyanin consisted of two parts. In the first he completed his determinations of the physical and chemical constants of the haemocyanin of the Australian crayfish. In the second he carried out an extensive study of the supposed oxidation of haemocyanin to methaemocyanin, using both electrometric titrations and determinations of magnetic susceptibility. After numerous experiments he came to the conclusion that such an oxidation has not been conclusively demonstrated. A paper on this work is awaiting publication. Since January he has devoted his time to a study of determinations of arsenic, and is endeavouring to improve on the existing rather cumbersome methods for its extraction from biological fluids.

Dr. Gottschalk has continued his determinations of the complete carbohydrate balance of the yeast cell under strictly anaerobic conditions, and under conditions of an ample supply of oxygen. He has shown that no more reserve carbohydrate is synthetized by the yeast cell from added glucose under aerobic than under anaerobic conditions. With fructose the same result was obtained and, in addition, it was found that the residual hexose in the fluid was fructose. These results are incompatible with the "carbohydrate cycle" proposed by Meyerhof as an explanation of the Pasteur effect. In contact with compressed yeast in the presence of oxygen acetaldehyde is oxidized to CO_2 and water to the extent of 43%. For each molecule so oxidized one is converted to ethyl alcohol by a coupled reduction. The remaining 14% is dismuted to acetic acid and ethyl alcohol. The velocity of oxidation of acetaldehyde is the same as that of glucose. This indicates that it is the substrate of the oxidizing enzyme system of the yeast, just as it is of the reducing enzyme. These results prove that even in the presence of oxygen the reoxidation of the reduced cozymase is almost exclusively effected by acetaldehyde. It must be concluded from this fact that the velocity of the reoxidation of the reduced cozymase by molecular oxygen within the yeast cell is much less than by the acetaldehyde acting as a hydrogen acceptor. Thus the Pasteur effect is due to the fact that in the presence of oxygen part of the cozymase is put out of action by persisting in the reduced state. That in turn diminishes the gross rate of carbohydrate utilisation, since the reoxidation of the reduced cozymase is the limiting factor in the speed of aerobic as well as of anaerobic fermentation of glucose. The relative velocities of the oxidation of acetaldehyde, ethyl alcohol, and acetic acid by the oxidising system of yeast decrease in this order. From this fact it is concluded that the first step in the oxidation of acetaldehyde is not, as is usually assumed, to acetic acid, but is more probably an oxidation leading to glycollic aldehyde. Dr. Gottschalk and Mr. Holden are investigating further this aspect of the problem.

H. F. Holden:

''Ultra-violet Absorption of Some Metallo-porphyrins and of their Compounds with Globin,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1941, 19, 2.

W. A. Rawlinson:

''The Variation of the Magnetic Susceptibility of Haemin in Various Solvents,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1940, 18, 185.

''The magnetic Properties of Potassium Molybdocyanide and Potassium Molybdicyanide,'' ''Journal of the Australian Chemical Institute,'' 1941, 8, 42.

E. Gottschalk:

''The Mechanism of the Pasteur Effect in Alcoholic Fermentation by Living Yeast Cells'' (in the press).

CLINICAL RESEARCH.

Alkalization of Urine.

In the treatment of haemolysis following incompatible blood transfusion rapid alkalization of the urine is of the first importance. It was found that the injection of 10 c.c. of a saturated sodium bicarbonate solution quickly caused this change, but the alkalization thus produced was not maintained. The injection of the same volume of 2 Molar sodium lactate solution slowly produced a more lasting effect. However, after the injection of 20 c.c. of a mixture of equal volumes of saturated sodium bicarbonate solution and 4 Molar sodium lactate solution the urine was found to be alkaline in fifteen minutes, and remained so for at least ninety minutes. The injection of this solution is therefore advocated on the first appearance of any symptoms suggestive of the occurrence of haemolysis following transfusion.

Serum in the Treatment of Shock and Haemorrhage.

Serum has been prepared from blood collected from Red Cross Voluntary Donors, and used in the treatment of cases of shock and haemorrhage both in its original form and after it has been dried and reconstituted. Serum has some advantages over blood; it can be stored for much longer periods, and it is unnecessary to keep it at refrigerator temperature. Dried serum will keep almost indefinitely. Since the agglutinin titre of the serum is greatly reduced by pooling, preliminary typing of the patient is unnecessary.

The serum heated to 37° C. was dried by vacuum distillation of water from it at the rate of 80-90 c.c. per hour. This method, however, is not recommended for the production of large amounts of dried serum, but is an inexpensive practical method of producing experimental quantities. The product obtained is yellow, coarsely granular, and a small portion of the protein is denatured so that after reconstitution the fluid is slightly turbid. Experiments are at present being carried out on the Hardyization of serum after preliminary concentration in cellophane tubes. The dried serum obtained by this method is a fine white powder which goes into solution very rapidly.

In the somewhat limited number of patients to whom serum has been administered the incidence of reactions has not been higher than after the transfusion of fresh blood. Our results, in agreement with those of other workers, indicate that for all cases of shock in which there has been little or no blood loss (e.g., burns, crushing injury, etc.) serum has some advantages, but that in the treatment of haemorrhage it should be administered only when fresh blood is not immediately obtainable.

Purification of Water.

In order to prevent reactions after intravenous therapy, pyrogen-free water must be used in the manufacture of the solutions. Such water is usually prepared by distillation in glass stills. It has been claimed that treatment of water with charcoal removes the pyrogenic substances. This work has been repeated using rabbits for preliminary tests. The adsorption of pyrogens to charcoal appears to depend upon the amount of charcoal used, upon adequate mechanical agitation, and upon the concentration of ionized salts in the water. The pH is of less importance. Filtration through Seitz E.K. filters was ineffective in removing pyrogens.

In clinical tests tap water shaken for 15 minutes with two parts per thousand of charcoal, filtered through filter paper and used to make saline solution which was autoclaved and injected intravenously gave only slight reactions. It is hoped to eliminate these by using a larger amount of charcoal and by more prolonged shaking.

Investigation of Sulfathiazole.

At the beginning of 1941 the firm of May and Baker supplied the Institute with tablets of sulfathiazole (M. & B. 760) for experimental test. Overseas experiments had given promise that this drug might be effective in staphylococcal and gonococcal infections. A variety of cases has been treated at the Royal Melbourne and Children's Hospitals, and estimations of the blood level and urinary excretion of the drug have been made. Large doses are necessary, but the preparation appears to be very well tolerated, rarely causing vomiting, and no ill effects on the kidneys or bone marrow have been detected. The soluble drug for intravenous use is not yet obtainable in Australia. It was, however, found possible to suspend the crushed tablets in olive oil for intramuscular use. The injection is painless, and produces no local reaction. Absorption is slow and prolonged. The results so far obtained in gonococcal and pneumococcal infections are encouraging.

MARJORIE BICK and E. B. DREVERMANN:

> "A Method for the Rapid Alkalization of Urine," "Medical Journal of Australia," 1941, 2, 223.
>
> "The Use of Pooled Human Serum for the Treatment of Haemorrhage and Shock" (in the press).

NEURO-PATHOLOGICAL RESEARCH.

Dr. E. Graeme Robertson has continued his pathological studies of material from cases of nervous disease which have been fully investigated clinically. He has studied a series of interesting vascular lesions, including one of thromboangeitis obliterans. He has also had the opportunity of investigating two rare tuberculous conditions—one a myelitis and the other in a patient with myxoedema who exhibited grasp reflexes which disappeared when appropriate thyroid treatment was instituted, but who finally succumbed with extensive tuberculous lesions.

Dr. Robertson's chief work in the period under review has been the completion of the monograph on encephalography to which reference was made in last year's report.

THE BLOOD BANK.

The blood bank, which was established in 1939 by Major Ian Wood and Miss F. E. Williams, is continuing under the charge of Miss Bick to supply blood for emergency transfusions to this and other metropolitan hospitals, as well as to doctors in private practice. In addition serum has been made available for cases of post-operative and burn shock. The average weekly supply of blood for emergency transfusion is 7 pints, and the average duration of storage 7 to 10 days. During the last year 562 donors of the Red Cross Voluntary Transfusion Service have been bled, 200 of these for serum, to supply the needs of the bank. The bank has supplied material for the experimental work on the concentration and chemical drying of serum and on the pharmacologically active substances in serum, described elsewhere in this report.

The conditions under which excessive fat is present in serum have been investigated. By examination of the serum of volunteers at intervals after breakfasts of various kinds we found, as have others, that after a high-fat meal fat was detectable for six hours or more, and that even minimal amounts of fat may cause clouding three or four hours after the meal.

We are indebted to the Australian Red Cross Society (Victorian Branch) for the provision of all the equipment used in this service. Our thanks are due also to Dr. Ella Macknight and Dr. Dorothy Synan, honorary medical officers to the blood bank, and their assistants, and to the donors of the Red Cross Voluntary Transfusion Service, whose loyal co-operation has enabled the bank to continue and extend its service to the community.

RED CROSS BLOOD TRANSFUSION SERVICE.

The Institute has again during the past year provided facilities for examination of donors for this service. Volunteers for the permanent section (i.e., those willing to answer calls both for transfusions for individual civilian patients and for the blood bank, or for blood for preparation of stored serum or testing serum for military use) have been medically examined at the Institute by Dr. Lucy Bryce, Captain Drevermann, Dr. Margaret Henderson, Dr. Fay Kinross, and medical officers of the public hospitals, with assistance from Miss Splatt, Dr. Jakobowicz, and Miss Bick.

Volunteers for the Emergency Section (i.e., those giving blood for military purposes only) have been examined chiefly by medical women, assisted by science graduates or other voluntary helpers in their own surgeries, or at the Red Cross rooms at No. 12 Collins Street.

The laboratory tests on volunteers for both sections of the Service have been done almost entirely at the Institute. Dr. Jakobowicz has been responsible for the blood group and sub-group tests, serum titre, haemoglobin estimations, and Kahn tests, Miss Williams and Dr. Bryce for the Wassermann reactions.

At the time of publication of the annual report of the Institute for 1939-40 the number of calls received by the Service since its inception in 1929 was 3411. At the end of March, 1941, it had risen to 6366. This large increase during the past year has been almost entirely due to military needs. To keep pace with it 2647 new volunteers have been enrolled and tested during the year, 1345 of them since an appeal for donors made in January, 1941. In addition to these new volunteers 280 donors who have given their blood repeatedly, or who were enrolled prior to the adoption in 1939 of serum titre estimations as a routine, have been re-examined.

During recent months for economy of time and material the Kahn test has been used instead of the Wassermann reaction for emergency donors. In the preparation of antigen and pure cholesterol, Mr. Holden has given most helpful co-operation. In a few cases giving doubtful reactions Wassermann tests have also been made.

Estimations of the agglutinin content of individual components and the final product of 181 samples of pooled serum for storage (169 prepared at the Commonwealth Serum Laboratories and 12 by Miss Bick) have also been made. This work has demonstrated that appropriate pooling of serum of different blood groups results in reduction of agglutinin titre to a very low level. The risk of dangerous interaction between agglutinins in suitably pooled stored serum and the cells of the recipient is therefore negligible.

The estimation of agglutinin titre has been essential also to enable us to select persons with high titre serum as donors for the testing serum prepared at the Commonwealth Serum Laboratories in large quantities for blood group tests for the Defence Forces. This serum is made available throughout the Commonwealth, but owing to the situation of the laboratories, the onus of providing it necessarily devolves on Melbourne donors.

The actual number of tests involved in the work of the Transfusion Service during the past year has been 2647 blood group tests, 945 sub-group tests, 2927 serum titre estimations, 2647 haemoglobin estimations, 2225 Wassermann tests (1096 by Miss Williams, 1229 by Dr. Bryce), and 534 Kahn tests. This rapidly increasing volume of work has placed a great strain on the personnel, space and equipment available. A junior technician and much necessary equipment have been provided by the Red Cross Society, but again this year the technical staff of the Institute, under the capable leadership of Miss Helen Wischusen, has given invaluable overtime voluntary assistance, and Mrs. Loudon has been responsible for the purchase of equipment and for the book-keeping.

L. M. Bryce and R. Jakobowicz:
''The Estimation of the Iso-agglutinin Titre of Human Serum and its Practical Applications,'' ''The Medical Journal of Australia,'' 1941, 1, 290.

R. Jakobowicz and L. M. Bryce:
''The Iso-agglutinin Titre of Pooled Serum or Plasma,'' ''The Medical Journal of Australia,'' 1941, 1, 318.
''Victoria and the Australian Red Cross Blood Transfusion Service,'' Leading Article, ''Medical Journal of Australia,'' 1941, 1, 363.

WAR WORK.

A large amount of work, principally of a routine nature, has been carried out by the staff of the Institute and of the Hospital routine services. Dr. F. M. Burnet and Mr. Holden have advised upon a number of problems. Miss Williams and Mrs. H. J. Gardner have tested a number of disinfectants for the Army and for the Medical Equipment Committee. Two hundred and nine members of the A.I.F., A.A.N.S., and 64 officers and other ranks of the A.A.M.C. were vaccinated and inoculated with T.A.B. and tetanus prophylactic, and their blood groups determined. Nearly 1000 sterility tests on catgut have been carried out for the services, and over 42,000 blood grouping tests have been carried out on members of the A.I.F. and R.A.A.F. In this work we have had the invaluable voluntary assistance of Dr. Rachel Gross, Mrs. Wilfred Evans, Dr. Phyllis Tewsley, Dr. Margaret Ashton, Miss Maud Cowen, Miss Mavis Swan, and Miss Winifred Amos.

Three medical officers have received special training in clinical pathology and biochemistry, and four other ranks have been or are being trained in laboratory technique for service overseas or at home.

Thirty-six medical students offered themselves as volunteers for a test of the immunising value of two ''cocktail vaccines'' prepared by the Commonwealth Serum Laboratories. One of these consisted of heat-killed B. typhosus and B. paratyphosus A and B with formalinized tetanus toxoid and the other of formalinized T.A.B. with tetanus toxoid. The subjects of the experiment were injected by Miss Williams and bled at appropriate intervals, and the titre of tetanus antitoxin and of typhoid and paratyphoid agglutinins determined at the Commonwealth Serum Laboratories. The cocktail vaccine containing formalin-killed T.A.B. was discarded because it caused several unpleasant reactions, and further large scale tests of the effectiveness of the other vaccine are now being carried out by the Army. The reactions observed with the completely formalinized cocktail vaccine, which occurred after the second injection, resembled the shock produced by the intravenous injection of typhoid vaccine, though they were somewhat slower in onset, rather than the anaphylactic reactions infrequently observed after second doses of tetanus toxoid alone.

During the course of the immunization of members of the A.A.M.C. and A.A.N.S. Dr. Bryce has taken the opportunity of studying the incidence of allergic sensitivity to tetanus toxoid, as demonstrated by intradermal tests, and its relationship to subsequent generalized reactions. For this purpose intradermal tests with diluted toxoid, broth controls, and in suitable cases other constituents of the toxoid medium supplied by the Commonwealth Serum Laboratories were also tested. None of these individuals showed sensitivity to the skin tests before the first dose of toxoid, and none suffered generalized reactions. The skin tests made prior to the second injection appeared to demonstrate slight sensitivity to the toxoid itself in three individuals in whom the interval between the two injections was only three weeks. Two of these nurses were given adrenalin with the subsequent immunizing injection; the third, whose skin reaction was very slight, was given toxoid alone. None had general reactions. In two other nurses skin tests made six weeks after the first injection of toxoid were strongly positive. In these the allergen appeared to be a constituent of the medium rather than the toxoid itself. In one of these simultaneous administration of adrenalin with the subsequent immunizing dose of toxoid failed to prevent a generalized reaction, for the relief of which further adrenalin was necessary. In the other case the toxoid was given in small amounts at approximately hourly intervals, and no general reaction occurred. Blood samples taken three weeks later from these two nurses showed satisfactory anti-toxic titre when tested at the Commonwealth Serum Laboratories.

In view of the report of the occurrence of similar symptoms, possibly due to allergy, after the first immunizing dose of toxoid in two soldiers, both of whom had received anti-tetanic serum a few months previously, skin tests have also been made in 79 individuals who had been given anti-tetanic serum approximately three months earlier. In two there were mild skin reactions. These, however, occurred with broth controls as well as with toxoid, and in any case were not at all comparable in intensity with the skin reactions given by persons known to be sufficiently sensitive to suffer general reactions to immunizing doses. In this small series, therefore, there is no evidence that sensitivity to toxoid, demonstrable by skin tests, is acquired as a result of previous administration of anti-tetanic serum.

ROUTINE HOSPITAL SERVICES.

Bacteriology and Clinical Pathology.

The routine work in this department is still increasing, but in addition to this a number of special investigations have been carried out.

During the last three years all cases of pneumonia in the Hospital have been typed. In 1938 and 1939 Type III was the predominant type, but this year Type V has been the most frequently encountered (20%), and Type I has for the first time also become frequent (17%).

The determination of prothrombin times has been done as a routine in all cases of jaundice, but very few have been found to fall below 30-35%, at which level the risk of haemorrhage becomes great.

In the allergic clinic Dr. Gardner has successfully treated a striking case of emotional urticaria by desensitization with Doryl. The patient has now gone through two summers without recurrence. During the last year many extracts have been prepared in this department for therapeutic use in allergic patients.

The efficiency of a number of disinfectants has been tested.

HILDA J. GARDNER:

> "A Fatal Case of Classical Weil's Disease (Leptospirosis icterohaemorrhagica) in Melbourne," "Medical Journal of Australia," 1940, 2, 310.

BIOCHEMISTRY AND BASAL METABOLISM.

In this department, in addition to the constantly increasing volume of routine work, some special investigations have been carried out.

Studies on liver function, using the hippuric acid and the Takata-Ara tests, are being continued. The sodium, potassium, and chloride content of the serum is being determined in a number of cases of adrenal disease, and the effects of treatment with percortin are being observed. The response of patients with achlorhydria to treatment with vitamin B_1 is being carried out under Dr. Ivan Maxwell's direction. Reference was made in last year's report to the results of studies on changes in the mineral content of the plasma in shed blood.

BERYL SPLATT:

> "Some Observations on Changes in the Mineral Content of Plasma and Serum," "Medical Journal of Australia," 1940, 2, 363.

MORBID ANATOMY.

During the year 510 autopsies were performed. Dr. C. H. Mollison and Dr. R. J. Wright-Smith were responsible for the examination of 1661 biopsy sections from 1246 specimens. Sections taken from autopsy material numbered 715. Routine examination of the appendix removed at operation was carried out on 443 occasions. The photography has been done by Miss Helen Wischusen.

MUSEUM.

New specimens added to the Museum included examples of endothelioma of the ileum, acute colitis, lipoma of the caecum, sarcoma of the male breast, adenoma of the parathyroid, actinomycosis of the lung and kidney, carcinoma of the fallopian tube, tuberculosis of the knee joint, and the stomach from a case of formalin poisoning. The remounting of old specimens has continued.

Specimens are being used more widely by members of the honorary staff, by graduates, and students.

TEACHING.

Dr. Wright-Smith has given the usual course of tutorial lectures in pathology to fourth year students in December and February. Lecture demonstrations in morbid anatomy have been given once weekly to fifth and sixth year students, and lectures in elementary bacteriology have been given to the student nurses in the Hospital. Tuition in clinical pathology was given to fifth year medical students by Dr. H. Gardner.

LIBRARY.

Our thanks for the gifts of journals and books are due to the following:—L'Académie Royale de Médecine de Belgique; The Commonwealth Department of Health; The Council of Scientific and Industrial Research; Miss Danks; Mr. Dobell, F.R.S.; The

London Hospital; The Medical Research Council; The Middlesex Hospital Medical School; New York State Department of Health, Division of Laboratories and Research; Department of Pathology University of Oxford; Rockefeller Institute, New York; Dr. R. J. Wright-Smith; La Société Royale des Sciences Médicales; The South African Institute for Medical Research; The University of Harvard, Department of Tropical Medicine; The University of Pennsylvania, Department of Pathology; The Government Institute for Infectious Diseases of the Tokyo Imperial University; The University of Tomsk.

CHARLES H. KELLAWAY,

Director.

The Walter and Eliza Hall Institute of Research in Pathology and Medicine

FINANCIAL STATEMENT FOR THE YEAR ENDED 30th JUNE, 1941.

INCOME ACCOUNT.

RECEIPTS.

To Balance at 30/6/40				£3,859	19	2
,, Trustees, W. & E. Hall Trust	£3,200	0	0			
,, University of Melbourne	750	0	0			
,, Felton Bequests Committee	270	0	0			
,, Estate Anthony Mackie	150	0	0			
,, Estate Alfred Edments	100	0	0			
,, Royal Australasian College of Surgeons	100	0	0			
,, Sir Harry Brown	5	5	0			
,, Royal Melbourne Hospital	404	13	8			
,, Interest on Investments	65	11	8			
,, Army and Air Force—Blood Typing	1,342	13	3			
,, Fees Received for Tests	336	18	9			
				6,725	2	4
				£10,585	1	6

EXPENDITURE.

By Salaries and Wages	£4,175	13	8			
,, Materials	408	13	3			
,, Apparatus	53	13	10			
,, Printing, Stationery, Postages, etc.	179	9	4			
,, Publications	141	19	8			
,, Fittings and Equipment	8	12	6			
,, Repairs to Apparatus	5	17	11			
,, Repairs to Buildings	15	13	1			
,, Bio-chemical Department	315	1	6			
				£5,304	14	9
,, Balance—						
Investments	1,875	0	0			
Cash — Agent-General, London	52	7	7			
Secretary's Advance A/c, E.S. & A. Bank	100	0	0			
Bank of N.S.W.	3,252	19	2			
				5,280	6	9
				£10,585	1	6

THE ELSIE MARION CARTY ACCOUNT.

To Balance at 30/6/40		£338 8 0	By Materials	£16 3 4	
,, Estate E. M. Carty	107 8 0		,, Grant, Virus Research	57 6 4	
,, Interest on Investments	167 12 0		,, Salaries	229 0 0	
		275 0 0			302 9 8
			,, Balance		310 18 4
		£613 8 0			£613 8 0

BIO-CHEMICAL DEPARTMENT ACCOUNT.

To Interest	£359 18 6	By Salaries	£675 0 0
,, Income Account	315 1 6		
	£675 0 0		£675 0 0

LIBRARY ACCOUNT.

To Interest	£83 9 0	By Books, Journals, and Bookbinding	£80 18 0
		,, Balance, Bank of N.S.W.	2 11 0
	£83 9 0		£83 9 0

VIRUS ACCOUNT.

To Balance at 30/6/40	£593	3	4	By Materials	£1,142	15	7
,, Commonwealth Government	1,565	0	0	,, Salaries and Wages	1,192	15	4
,, Mr. E. Alec Cato	1,000	0	0	,, Balance, Bank of N.S.W.	822	12	5
	£3,158	3	4		£3,158	3	4

ARMY MEDICAL RESEARCH FUND.

To Mr. A. P. Adams	£1,000	0	0	By Materials	£31	15	5
				,, Institute of Medical and Veterinary Science, Adelaide	112	16	8
				,, Salary	61	0	2
				,, Balance, Bank of N.S.W.	794	7	9
	£1,000	0	0		£1,000	0	0

ENDOWMENT FUNDS.

To Bio-chemical Endowment Fund	£8,612	10	3	By Investments	£14,899	3	10
,, Elsie Marion Carty Fund	4,531	3	6	,, Current A/c, Bank of N.S.W.	274	9	11
,, Library Fund	2,030	0	0				
	£15,173	13	9		£15,173	13	9

NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL GRANTS.

To Balance at 30/6/40	£213	14	3	By Salaries and Wages ..	£3,065	13	11			
,, Commonwealth Government	3,395	14	10	,, Materials	289	12	7			
				,, Balance, Bank of N.S.W.	254	2	7			
								£3,609	9	1
	£3,609	9	1					£3,609	9	1

AUDITOR'S CERTIFICATE.

I have to report that I have completed the Audit of the Books and Accounts of the Institute for the period ended 30th June, 1941. I have verified all receipts and have had vouchers produced for all disbursements. All information and explanations required have been given. The Statement is a correct statement of Receipts and Expenditure as revealed by the books of the Institute.

15th July, 1941. (Signed) W. M. JARVIE, F.C.A. (Aust.), Auditor.